

Objection to Claim 7:

This object is believed to have been obviated by appropriate amendment to Claim 1. As amended, Claim 1 now recites "nucleic acids," rather than "DNA." Thus, the limitation presented in Claim 7 to RNA is now appropriate.

Rejection of Claims 1-29 Under 35 USC §112, First Paragraph:

This rejection is believed to have been overcome, in part, by appropriate amendment to the claims, and is, in part, respectfully traversed.

This rejection is believed to have been overcome, in part, by amending the claims to remove the passage indicating that the randomized portion can be "anywhere within" the region of fixed nucleotide sequence. The claims have also been amended to insert a lower limit (10 nts) for the overall length of the various primers.

The remainder of this rejection is respectfully traversed.

With respect to the number paragraphs 1-9, beginning on page 3 of the Office Action, each one of these concerns is exceedingly well known to one of ordinary skill in the art. Hence, these issues, while they must be taken into account, must be taken account of in ANY PCR reaction. That does not render the PCR unenabled. It simply means that the PCR is empirical in nature. Specifically:

1. The purity of the nucleic acid preparation. Isolating DNA is extraordinarily well known. Sambrook, Fritsch and Maniatis (a 1989 prior art laboratory procedures manual), cited in the application at page 20, lines 1-9, exhaustively describes how to isolate DNA so that it is sufficiently pure to undergo the PCR.
2. GC content of the probes and target. The application directly addresses this point at page 23, lines 17-29. Determining GC content and calculating the melting point of a double-stranded DNA based on its GC content is well known in the art.
3. Length of primers. This issue has been addressed by appropriate amendment to the claims. The lower limit of the primers is now explicitly noted as being 10 nts or greater.

4-9 Each of these concerns: ionic strength, incubation temperature, incubation time, target and primer concentrations, presence of denaturing agents, and volume exclusion agents, are all well known in the art.

The Office recites this very large list as if each parameter were unknown or as if addressing each parameter were *de novo* investigation. A patent application, however, is not addressed to the rank amateur. It is addressed to the person of ordinary skill in the art. Thus, there is long-standing precedent holding that a patent application need not describe that which is common and well known in the art. All of the concerns listed in the numbered paragraphs are not only known in the art, but there is a broad swath of laboratory literature of how to navigate through such concerns. Hence the phenomenal success of the Sambrook, Fritsch and Maniatis series of "Molecular Cloning" lab manuals.

Resolving any of these considerations, or all of them, or any sub-set of them, is well within the skill of the ordinary Ph.D. level molecular biologist. The target to be amplified is divided into aliquots, as are the primers to be used, and the PCR is run under a host of different conditions (ionic strengths, melt times, extension times, reanneal times, etc.) The conditions are optimized and the reaction is then performed again, under the optimized conditions. This is not undue experimentation. This is how all PCR reaction are run. This is the routine way of ensuring that the conditions under which the PCR is performed will yield the maximum amount of specific reaction product.

Regarding the Examiner's observation that the process would seem to result in a "hodgepodge of sequences of dubious characteristics" (Office Action, page 10, line 1), Applicant respectfully notes that Dr. Senapathy's previous Rule 132 Declaration can hardly be considered "dubious." The results presented therein are objective scientific results, generated using the method now recited. The Office is not free simply to label these results "dubious" without a more detailed explanation.

The Office also notes, at page 10, that "the specification, not the claims, recites the need for the concentration of primers to be increased 'many thousand fold' in order for the assay to proceed normally." The Office goes on to state that this limitation does not appear in the claims and therefore the claim is somehow unclear. Claims, however, are not read in

a vacuum. While limitations appearing in the specification are not to be "read into" a claim, nor are limitations that positively appear to be read out of a claim. In this regard, note that step (c) of Claim 1 explicitly requires:

- (c) **amplifying the nucleic acid template via the PCR using the plurality of first PCR primers and the plurality of second PCR primers under conditions wherein a subset of the plurality first primers binds to the consensus sequence of interest substantially wherever it occurs in the template, and a subset of the plurality of second primers binds to the template at locations removed from the first primers such that nucleic acid regions flanked by the first primer and the second primer are specifically amplified.**

Emphasis added. In short, the claims explicitly require that the conditions of amplification be such that specific amplification occurs. The concentration of the primers is empirically derived. As noted in the specification, the primer concentration may have to be large. Rather than rendering the claim ambiguous, or unenabled, the passage at page 9 of the specification clearly indicates to a person attempting to recreate the now-claimed invention, what must be done to get acceptable results. Applicants therefore submit that this portion of the rejection is clearly improper.

Returning back to the issue of Dr. Senapathy's declaration, there is not statute, rule, regulation, or MPEP statute stating that Rule 132 Declarations of an inventor are suspect or are somehow of a sub-standard nature. Granted, Dr. Senapathy surely is not a disinterested party. He's the inventor. But, his Declaration contains highly detailed, step-by-step instructions and results in the form of gels, sequence results, and the like. His Declaration clearly shows that the method of the present invention will yield sequence data. The Office is not free to ignore the substantive, objective scientific data contained in Dr. Senapathy's Declaration.

A close examination of the Declaration does reveal that the sequence is generated using primers having a fixed 3' terminus and a randomized 5' terminus. Thus, the declaration should be considered dispositive of this issue. The Office, however, has not articulated why it is of the opinion that the invention would not function with the same success having the randomized portion and the fixed portion at opposite ends of the primer. The primer will bind

wherever there is a sufficient match within the target nucleic acid, regardless of the location of the random and fixed portions of the primer. Once the primer is annealed to the target, the PCR proceeds in standard fashion. The Office Action has not addressed why it is of the opinion that the invention would not work otherwise. Applicant therefore respectfully submits that Dr. Senapathy's Declaration is, in fact, dispositive of the enablement and functionality of the claims as the now stand.

In light of the amendment to the claims, the above remarks, and Dr. Senapathy's earlier-submitted Rule 132 Declaration, Applicant submits that this rejection has been overcome. Withdrawal of the same is now requested.

Rejection of Claims 1-29 Under 35 USC §112, Second Paragraph:

This rejection is believed to have been overcome, in part, by appropriate amendment to the claims, and is, in part, respectfully traversed.

This rejection is believed to have been overcome, in part, by amending the claims to remove the passage indicating that the randomized portion can be "anywhere within" the region of fixed nucleotide sequence.

In response to the question at page 11 of the Office Action "...how can one perform any amplification when the first primer, and conceivably the second primer, have the 'identical' sequence...?" Applicant notes that the Office is reading the passage in a vacuum. The full passage notes that the sequence of both of the first or second primers may be "identical or complementary." Clearly, for a single-stranded target nucleic acid, the primers must be complementary. In a double-stranded target, sequence are identical to one strand, will, of course, be complementary to the other.

Regarding the nature of the second primer, Applicant notes that the concerns voiced by the Examiner are addressed both in the specification and in the Rule 132 Declaration of Periananan Senapathy, submitted earlier. The second primer is designed based on the probability of how often an oligonucleotide of given length will appear. As noted in Applicant's response to the Office Action dated March 31, 2001:

Any non-specificity can be avoided by fine-tuning the reaction conditions such as by adjusting the annealing temperature and the reaction temperature during amplification, and/or adjusting the length and G/C content of the primers. These adjustments are routinely done in the standard PCR amplification protocol. In short, although the partly-fixed primers have a random sequence component, a sub-population of the primer molecules will have the exact sequence that would bind with the exact target sequence. The proportion of the molecules with exact sequence that would bind with the exact target sequence will vary depending on the number of random characters in the partly-fixed primers. For example, a primer 11 nucleotides long with 6 characters fixed and 5 characters random, one in about 1000 primer molecules will have the exact sequence complementary to the target sequence on the template.

By increasing the concentration of the primers appropriately, a comfortable level of PCR amplification required for sequencing can be achieved. When primer concentration is increased, it requires an increase in the concentration of magnesium, which is required for the function of the polymerase enzyme. The excess primers (and "primer-dimers" formed due to excess of primers) can be removed after amplification reaction by a gel-purification step.

Any non-specific binding by any population of the primers to non-target sequences can be avoided by adjusting (increasing) the temperature of re-annealing appropriately during DNA amplification. It is well known that the change of even one nucleotide due to point-mutation in some cancer genes can be detected by DNA-hybridization. Hybridization is routinely used for diagnosing particular cancer genes (*e.g.*, John Lyons, "Analysis of *ras* gene point mutations by PCR and oligonucleotide hybridization," in PCR Protocols: A guide to methods and applications, edited by Michael A Innis et al., (1990), Academic Press, New York). This is done by adjusting the "re-annealing" or "melting-temperature", and fine-tuning the reaction conditions. Thus the binding of non-specific sequences even with just one nucleotide difference compared to the target binding-site in the template sequence can be avoided.

Applicant therefore submits that these concerns are directly addressed in the specification. Applicant also notes that the Rule 132 Declaration earlier submitted clearly shows that the invention generates sufficiently clean signal to enable nucleotide sequencing.

Also as noted earlier, the PCR is empirical in nature (notoriously so). Routine considerations that are addressed in every PCR protocol includes primer and target concentration, magnesium concentration, cycling parameters (and many others). But those

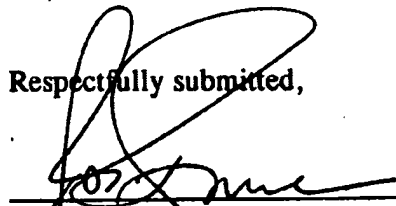
considerations are well known and routine. They are part and parcel of performing any PCR-based assay or protocol.

Therefore, it is respectfully submitted that the claims as they presently stand are in full compliance with 37 CFR §112, second paragraph. Withdrawal of the rejection is respectfully requested.

CONCLUSION

Applicant respectfully submits that the application is now in condition for allowance. Early notification of such action is earnestly solicited.

Respectfully submitted,


Joseph T. Leone, Reg. No. 37,170
DEWITT ROSS & STEVENS, S.C.
Firstar Financial Centre
8000 Excelsior Drive, Suite 401
Madison, Wisconsin 53717-1914
Telephone: (608) 831-2100
Facsimile: (608) 831-2106

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appln. Serial No.: 09/431,451

Group Art Unit: 1650

Filing Date: November 1, 1999

Examiner: Sisson, B.

Applicant: Senapathy, P.

Attorney Docket No.: 34623.005

Title: METHOD FOR AMPLIFYING SEQUENCES FROM UNKNOWN DNA

"MARKED UP" CLAIMS AS AMENDED, 37 CFR §1.121(c)(1)(ii)

1. **[AMENDED THREE TIMES]** A method of amplifying desired regions of nucleic acid from a nucleic acid template comprising:
 - (a) providing a plurality of first PCR primers, each first primer having an overall length of at least about 10 nucleotides and further having a region of fixed nucleotide sequence identical or complementary to a consensus sequence of interest and a region of randomized nucleotide sequence located 5' to, 3' to, [anywhere within,] or flanking the region of fixed nucleotide sequence;
 - (b) providing a plurality of second PCR primers, each second primer having an overall length of at least about 10 nucleotides and further having a region of arbitrary, yet fixed nucleotide sequence and a region of randomized nucleotide sequence located 5' to, 3' to, [anywhere within,] or flanking the region of fixed nucleotide sequence; and then
 - (c) amplifying the nucleic acid template via the PCR using the plurality of first PCR primers and the plurality of second PCR primers under conditions wherein a subset of the plurality first primers binds to the consensus sequence of interest substantially wherever it occurs in the template, and a subset of the plurality of second primers binds to the template at locations removed from the first primers such that [DNA] nucleic acid regions flanked by the first primer and the second primer are specifically amplified.

12. **[AMENDED THREE TIMES]** A method of amplifying exons from a [DNA] nucleic acid template comprising:
 - (a) providing a plurality of first PCR primers, each first primer having an overall length of at least about 10 nucleotides and further having a region of fixed nucleotide sequence identical or complementary to a consensus sequence of a 3' splice region and a region of randomized nucleotide sequence located 5' to, 3' to, [anywhere within,] or flanking the region of fixed nucleotide sequence;
 - (b) providing a plurality of second PCR primers, each second primer having an overall length of at least about 10 nucleotides and further having a region

of fixed nucleotide sequence reversely complementary to a consensus sequence of a 5' splice region and a region of randomized nucleotide sequence located 5' to, 3' to, [anywhere within,] or flanking the region of fixed nucleotide sequence; and then

- (c) amplifying the [DNA] nucleic acid template via the PCR using the plurality of first PCR primers and the plurality of second PCR primers under conditions wherein a subset of the plurality first primers binds to a sequence reversely complementary to the 3' splice consensus sequence substantially wherever it occurs in the template, and a subset of the plurality of second primers binds to the 5' splice consensus sequence substantially wherever it occurs in the template, such that exons flanked by the first primer and the second primer are specifically amplified.

19. **[AMENDED THREE TIMES]** A method of amplifying regions flanking a consensus sequence in a nucleic acid template of totally or partially unknown sequence comprising:

- (a) providing a plurality of first PCR primers, each first primer having an overall length of at least about 10 nucleotides and further having a region of fixed nucleotide sequence identical or complementary to a consensus sequence of interest and a region of randomized nucleotide sequence located 5' to, 3' to, [anywhere within,] or flanking the region of fixed nucleotide sequence;
- (b) providing a plurality of second PCR primers, each second primer having an overall length of at least about 10 nucleotides and further having a region of arbitrary, yet fixed nucleotide sequence and a region of randomized nucleotide sequence located 5' to, 3' to, [anywhere within,] or flanking the region of fixed nucleotide sequence; then
- (c) amplifying the nucleic acid template via the PCR using the plurality of first PCR primers and the plurality of second PCR primers under conditions wherein a subset of the plurality first primers binds to the consensus sequence of interest substantially wherever it occurs in the template, and a subset of the plurality of second primers binds to the template at locations removed from the first primers such that [DNA] nucleic acid regions flanked by the first primer and the second primer are specifically amplified; then
- (d) incorporating the amplified nucleic acid of step (c) into a library;
- (e) sequencing a portion of amplified nucleic acid from a particular clone from the library of step (d) and providing a third PCR primer of unique sequence and having an overall length of at least about 10 nucleotides which will prime PCR amplification from the sequenced portion of DNA;
- (f) providing a plurality of fourth PCR primers, each fourth primer having an overall length of at least about 10 nucleotides and further having a region of arbitrary, yet fixed nucleotide sequence and a region of randomized nucleotide sequence located 5' to, 3' to, [anywhere within,] or flanking the region of fixed nucleotide sequence; and then

- (g) amplifying the nucleic acid present in the template via the PCR using the third PCR primer and the plurality of fourth PCR primers under conditions wherein the third primer binds to the sequenced portion of nucleic acid from step (e), and a subset of the plurality of fourth primers binds to the template at locations removed from the third primers such that [DNA] nucleic acid regions flanked by the third primer and the fourth primer are specifically amplified.